Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Schuster SJ, Svoboda J, Chong EA, et al. Chimeric antigen receptor T cells in refractory B-cell lymphomas. N Engl J Med 2017;377:2545-54. DOI: 10.1056/NEJMoa1708566

Figure S1: Protocol Schema

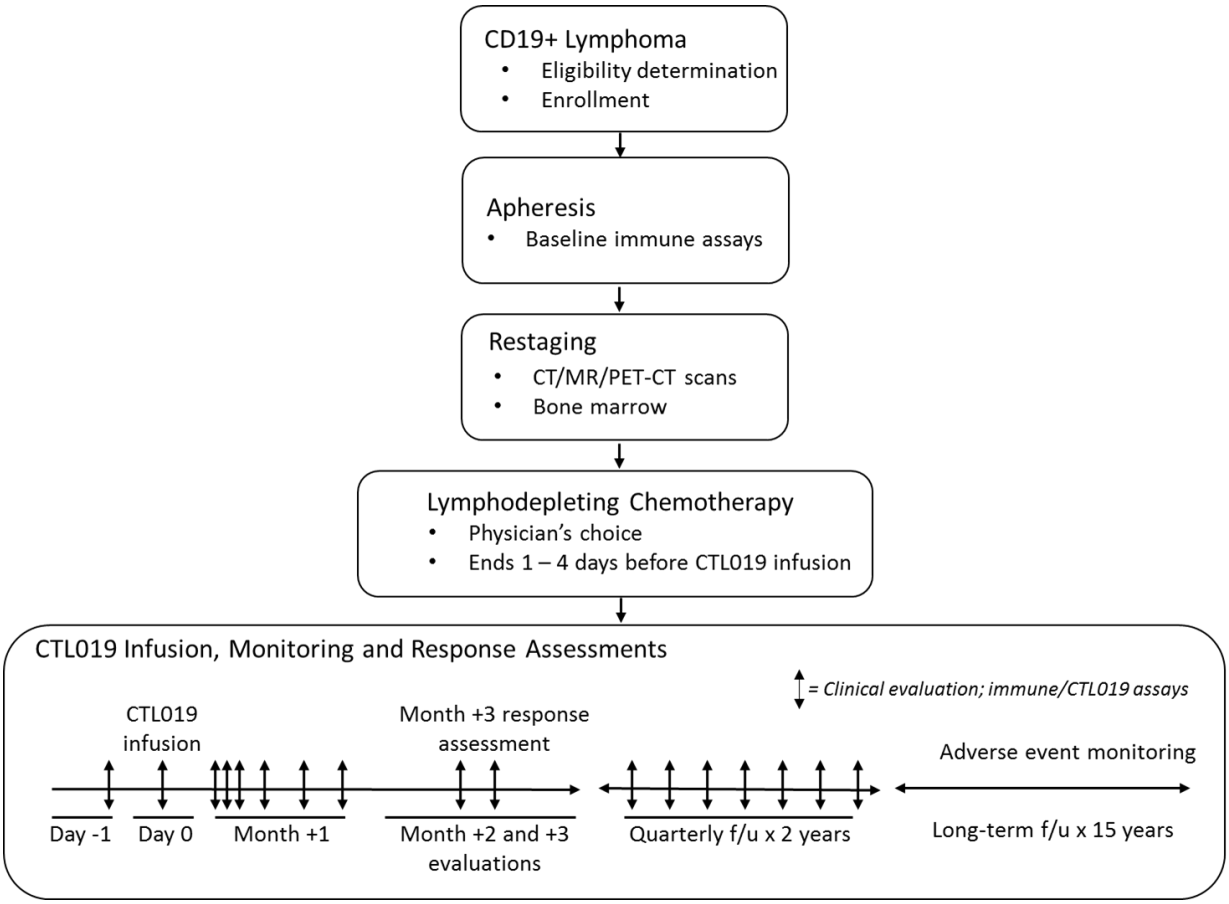


Figure S2: Patient Allocation

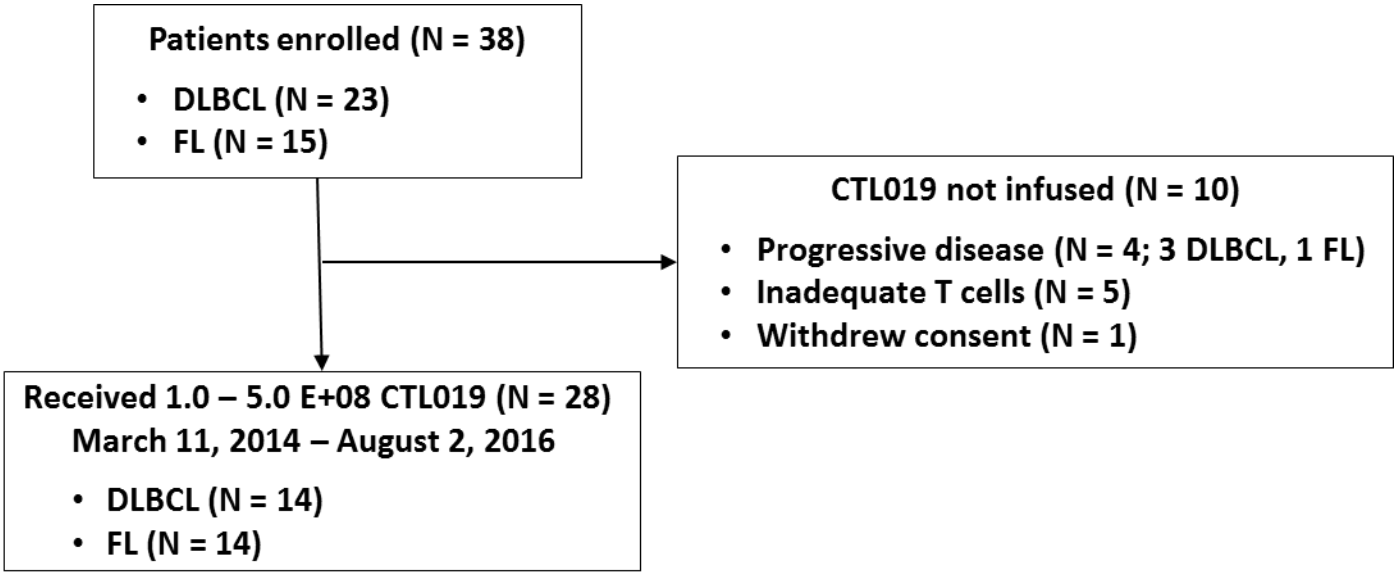


Table S1: Lymphodepleting Chemotherapy Regimens

Diffuse Large B-cell Lymphoma: Lymphodepleting chemotherapy (n = 14)

(n)	Regimen
6	hyperfractionated cyclophosphamide (1.8 gm/m^2)
2	modified EPOCH (doxorubicin 10 mg/m^2 and etoposide 50 mg/ m^2 daily x 4 by continuous infusion, cyclophosphamide 750 mg/m^2; no prednisone, no vincristine)
2	cyclophosphamide (1 gm/m^2)
2	bendamustine (90 mg/m^2 daily x 2)
1	radiation therapy (4000 cGy) + cyclophosphamide (750 mg/m^2)
1	infusional etoposide + bolus cyclophosphamide (etoposide 50 mg/ m^2 daily x 4 by continuous infusion, cyclophosphamide 750 mg/m^2)

Follicular Lymphoma: Lymphodepleting chemotherapy (n = 14)

(n)	Regimen
6	bendamustine (90 mg/m^2 daily x 2)
1	cyclophosphamide (200 mg/m^2) + fludarabine (20 mg/m^2) daily x 3
2	radiation therapy (400 cGy) + cyclophosphamide (1 gm/m^2)
1	radiation therapy (2200 cGy) + cyclophosphamide (750 mg/m^2)
1	cyclophosphamide (1 gm/m^2)
1	cyclophosphamide (1.2 g/m^2) over 4 days
1	carboplatin + gemcitabine (375 mg/m^2 + 750 mg/m^2)
1	modified EPOCH (doxorubicin 10 mg/m^2 and etoposide 50 mg/ m^2 daily x 4 by continuous infusion, cyclophosphamide 750 mg/m^2 ; no prednisone, no vincristine)

Table S2: Baseline Characteristics of All Patients

	Patients enrolled (N = 38)	Patients infused (N = 28)
Age, median	56.5y (range 25-77)	57.5y (range 25-77)
Female sex	15 (39%)	10 (36%)
Prior therapies, median	4 (1-10)	4.5 (1-10)
Advanced Stage (III-IV)	30 (79%)	21 (75%)
Bone marrow involved	8/36 (22%)	7/28 (25%)
Elevated LDH	26 (68%)	17 (61%)
ECOG PS, median	1 (range 0-1)	0 (range 0-1)
Prior autologous SCT	12 (32%)	10 (36%)
Prior allogeneic SCT	1 (3%)	1 (4%)

Table S3: All Adverse Events

Adverse Events (n)	Grade					Total Events
	1	2	3	4	5	
Blood and lymphatic system disorders Total	**2**	**6**	**7**	**1**		**16**
Anemia	2	6	3			11
Blood and lymphatic system disorders - Other			1			1
Febrile neutropenia			3			3
Leukocytosis				1		1
Cardiac disorders Total	**4**	**2**	**1**	**1**		**8**
Atrial fibrillation			1			1
Cardiac arrest				1		1
Chest pain - cardiac	1					1
Restrictive cardiomyopathy		1				1
Sinus tachycardia	3	1				4
Ear and labyrinth disorders Total	**3**					**3**
Hearing impaired	1					1
Vertigo	2					2
Eye disorders Total	**4**	**2**		**1**		**7**
Blurred vision	1					1
Conjunctivitis	1					1
Eye disorders - Other, specify		2				2
Floaters	2					2
Optic nerve disorder				1		1
Gastrointestinal disorders Total	**28**	**10**	**4**			**42**
Abdominal distension	3					3
Abdominal pain	1	3	1			5
Constipation	8	1				9
Diarrhea	6					6
Dysphagia	1					1
Gastrointestinal disorders - Other	1	1	1			3
Intra-abdominal hemorrhage			1			1
Mucositis oral	1	1				2
Nausea	5	3				8
Small intestinal obstruction			1			1
Vomiting	2	1				3
General disorders and administration site conditions Total	**29**	**14**	**3**			**46**
Chills	1					1
Edema limbs	5	2				7
Fatigue	12	5	1			18
Fever	3	3	1			7
Localized edema	1					1
Non-cardiac chest pain	1		1			2
Pain	6	4				10
Immune system disorders Total		**13**	**4**	**1**		**18**
Allergic reaction		2				2
Cytokine release syndrome		11	4	1		16
Infections and infestations Total	**6**	**30**	**7**	**1**		**44**
Abdominal infection		1				1
Bronchial infection		2				2
Gum infection	1	1				2
Infections and infestations - Other	4	6	1			11
Lung infection		1	4			5
Mucosal infection	1					1
Papulopustular rash		1				1
Rash pustular		1				1
Sepsis				1		1
Sinusitis		4				4
Skin infection			1			1
Small intestine infection			1			1
Upper respiratory infection		8				8
Urinary tract infection		4				4
Vaginal infection		1				1
Investigations Total	**35**	**25**	**20**	**12**		**92**
Alanine aminotransferase increased	8	2				10
Aspartate aminotransferase increased	4	1	1			6
Blood bilirubin increased	4	2				6
CD4 lymphocytes decreased		1	1			2
Cholesterol high	2					2
Creatinine increased	5	2				7
Lymphocyte count decreased		3	1	2		6
Lymphocyte count increased		1				1
Neutrophil count decreased	2	2	10	8		22
Platelet count decreased	5	2	2	1		10
Weight gain		1				1
Weight loss	3	1	1			5
White blood cell decreased	2	7	4	1		14

Adverse Events (n)	Grade					Total Events
	1	2	3	4	5	
Metabolism and nutrition disorders Total	**64**	**15**	**11**	**2**		**92**
Acidosis			3			3
Anorexia	1	1	1			3
Dehydration	1					1
Hypercalcemia	1	1		1		3
Hyperglycemia	15	3				18
Hypermagnesemia	1	1				2
Hypoalbuminemia	5	4				9
Hypocalcemia	8	3	1			12
Hypokalemia	9	1				10
Hypomagnesemia	9					9
Hyponatremia	13		2			15
Hypophosphatemia	1	1	4	1		7
Musculoskeletal and connective tissue disorders Total	**20**	**3**	**2**			**25**
Arthralgia	5					5
Back pain	8	1				9
Flank pain	1					1
Joint range of motion decreased			1			1
Musculoskeletal and connective tissue disorder - Other	2	1	1			4
Neck pain	2					2
Osteoporosis		1				1
Pain in extremity	2					2
Nervous system disorders Total	**17**	**5**	**4**	**1**	**1**	**28**
Cognitive disturbance	1					1
Dizziness	2					2
Encephalopathy			1	1	1	3
Headache	8	3				11
Hydrocephalus			1			1
Memory impairment		1				1
Movements involuntary	1					1
Nervous system disorders - Other	2					2
Paresthesia	1					1
Peripheral motor neuropathy		1				1
Spasticity			1			1
Syncope			1			1
Tremor	2					2
Psychiatric disorders Total	**2**	**6**	**2**			**10**
Anxiety		3				3
Confusion		1				1
Delirium		2				2
Hallucinations	2					2
Insomnia			2			2
Renal and urinary disorders Total	**6**		**3**			**9**
Acute kidney injury	1		2			3
Hematuria			1			1
Renal and urinary disorders - Other	2					2
Urinary frequency	2					2
Urinary urgency	1					1
Reproductive system and breast disorders Total	**1**					**1**
Perineal pain	1					1
Respiratory, thoracic and mediastinal disorders Total	**10**	**14**	**3**	**3**		**30**
Allergic rhinitis	1					1
Aspiration			1	1		2
Bronchial stricture		1				1
Cough	2	4				6
Dyspnea	1		1			2
Hoarseness	1					1
Hypoxia			1			1
Laryngeal edema				1		1
Nasal congestion	1	2				3
Pleural effusion		2				2
Pneumonitis		2				2
Pulmonary edema		2		1		3
Respiratory, thoracic and mediastinal disorders - Other	3					3
Retinoic acid syndrome		1				1
Sleep apnea	1					1
Skin and subcutaneous tissue disorders Total	**12**	**9**				**21**
Alopecia	2					2
Erythema multiforme	1					1
Erythroderma		1				1
Purpura	2					2
Skin and subcutaneous tissue disorders - Other	5	8				13
Skin ulceration	2					2
Vascular disorders Total	**1**	**5**	**5**	**1**		**12**
Hot flashes	1					1
Hypertension		2	3			5
Hypotension		1	2	1		4
Thromboembolic event		1				1
Vascular disorders - Other		1				1

Figure S3: Percentage CTL019 cells of CD3 cells over time post infusion by flow cytometry in DLBCL patients

A.

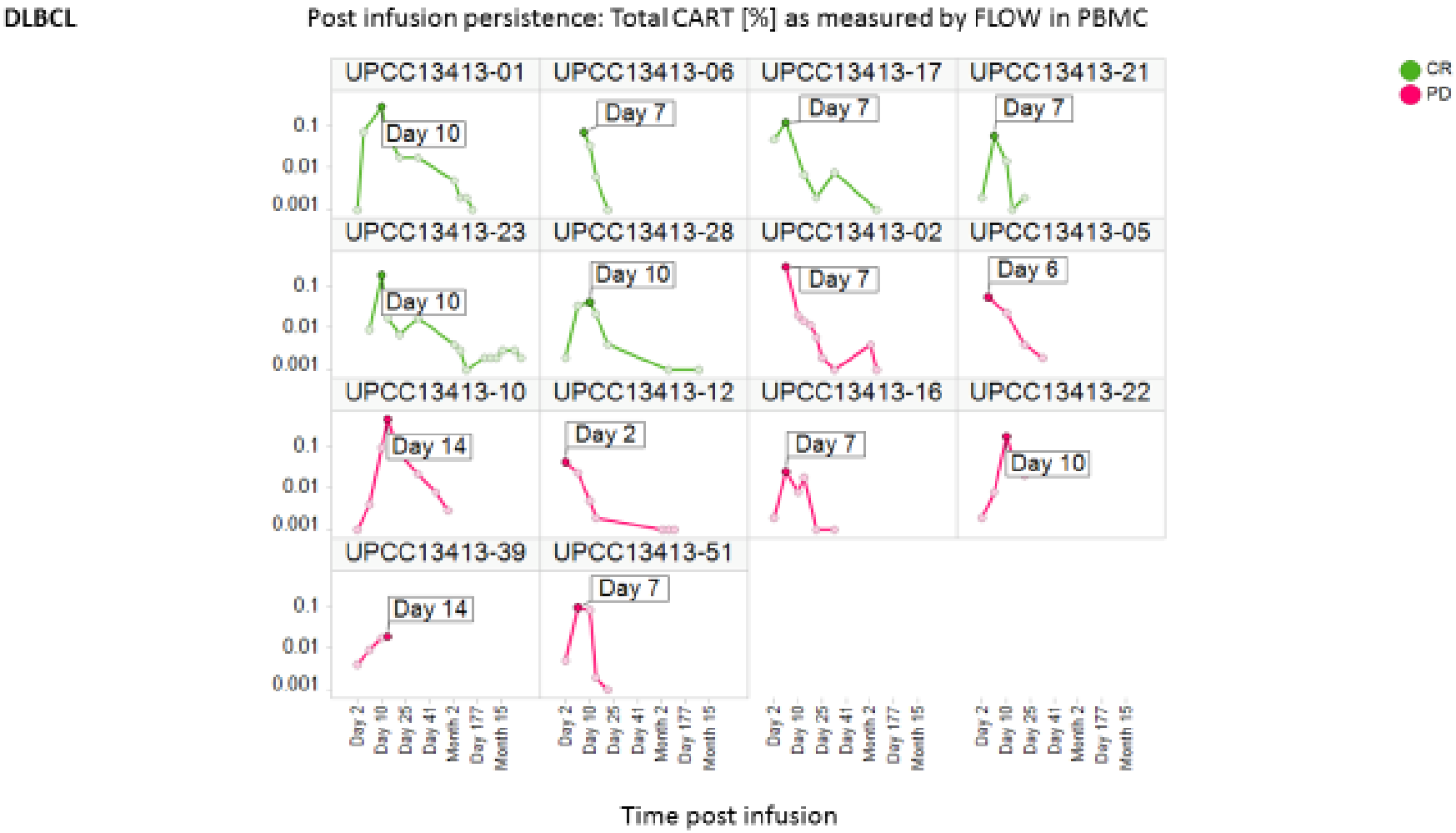


Figure S3: Percentage CTL019 cells of CD3 cells over time post infusion by flow cytometry in FL patients

B.

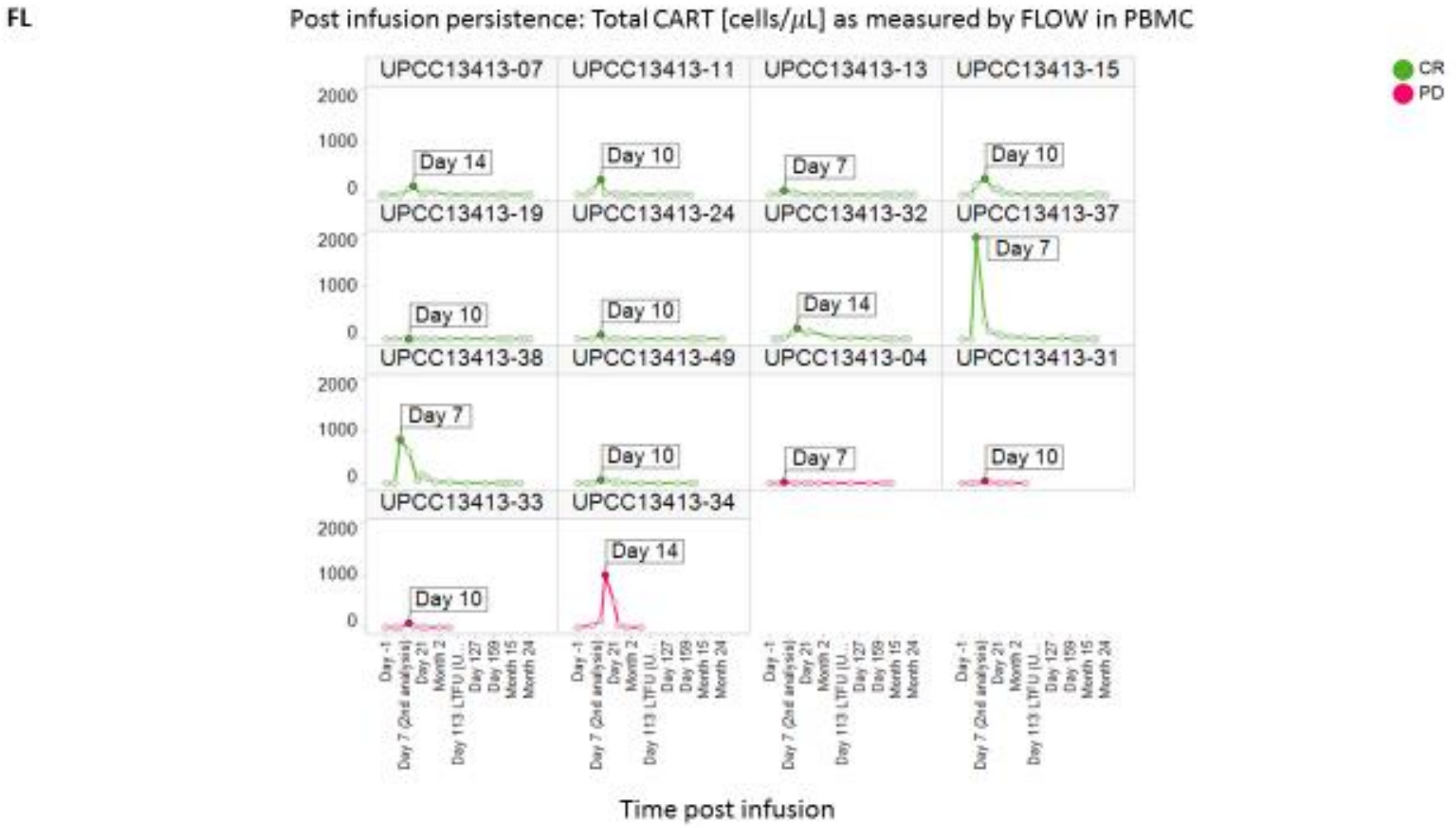


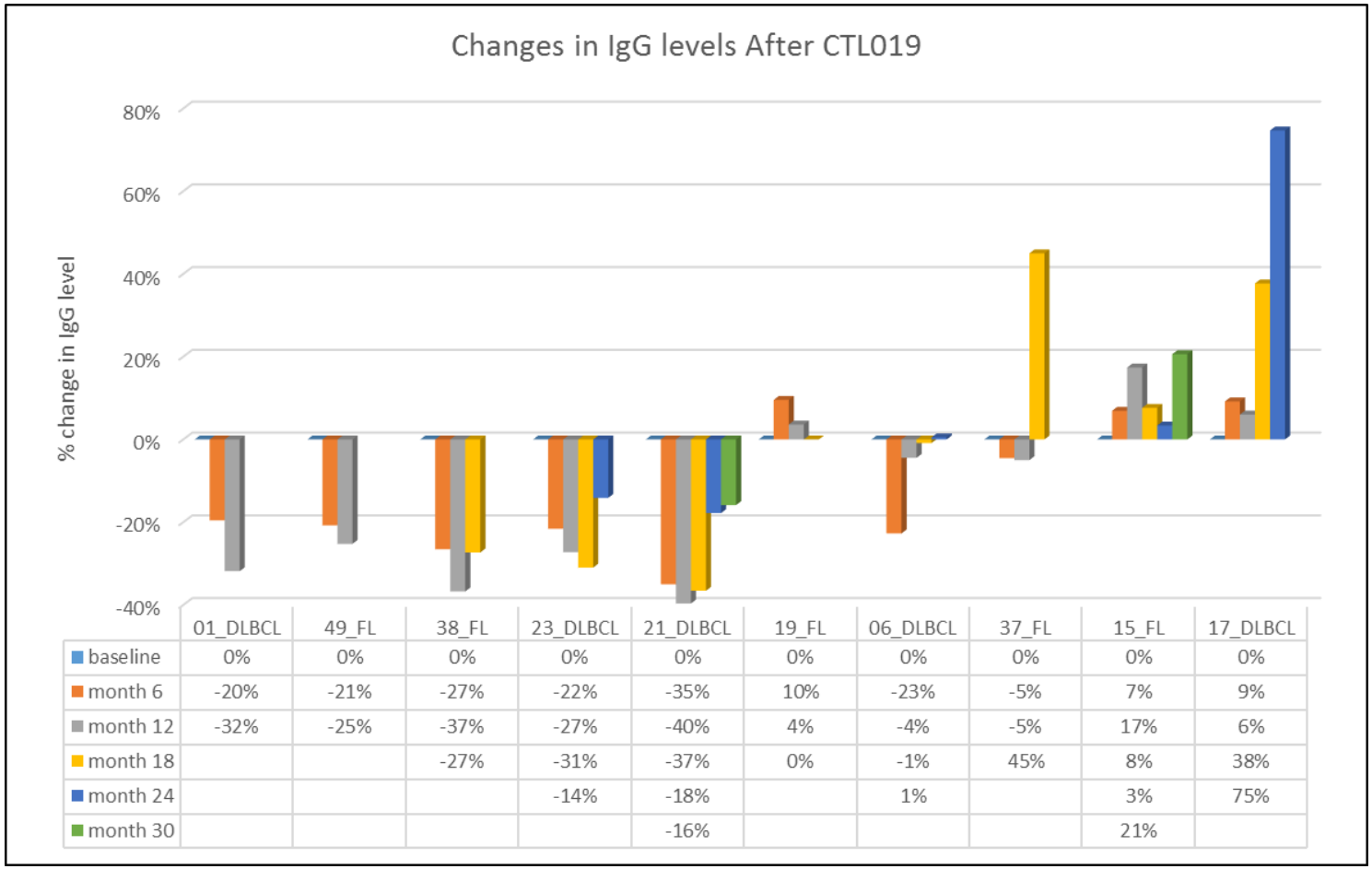
Figure S4: Changes in immunoglobulin levels over time in 10 patients in complete remission who were not receiving intravenous immunoglobulin.

Legend: Of 16 patients in complete remission at 6 months after CTL019, 2 patients were already receiving regularly scheduled intravenous immunoglobulin (IVIG) at enrollment and 2 patients with severe antecedent hypogammaglobulinemia (IgG levels <200 mg/dL) began IVIG less than 6 months after CTL019, one patient at 2 months following an episode of pneumonia and one patient at 5 months for prophylaxis. Twelve patients in complete remission at 6 months after CTL019 who were not receiving prophylactic IVIG administration were monitored for worsening hypogammaglobulinemia, frequency of infections, and immunoglobulin recovery. Two of these patients were started on IVIG for hypogammaglobulinemia with recurrent sinopulmonary infections at 12 and 22 months after CTL019; IgG levels in these patients had decreased by -66% and -13% from baseline levels, respectively.

Ten patients did not receive IVIG after CTL019 and we followed changes in immunoglobulin levels (data shown below). At 1 year, 3 patients maintained an IgG level within 5% of their pre-CTL019 level; 2 patients had increases of 6% and 17% above baseline IgG levels at one year with continued increases above baseline levels by 75% at 24 months and 21% at 30 months, respectively. Five patients had decreases in IgG levels from baseline by -25 to -40% at one year, although 2 patients with longer follow up appear to be improving (-27% to -14% and -40% to -16% at 24 and 30 months, respectively).

Figure S4: Changes in immunoglobulin levels over time in 10 patients in complete remission who were not receiving intravenous immunoglobulin.

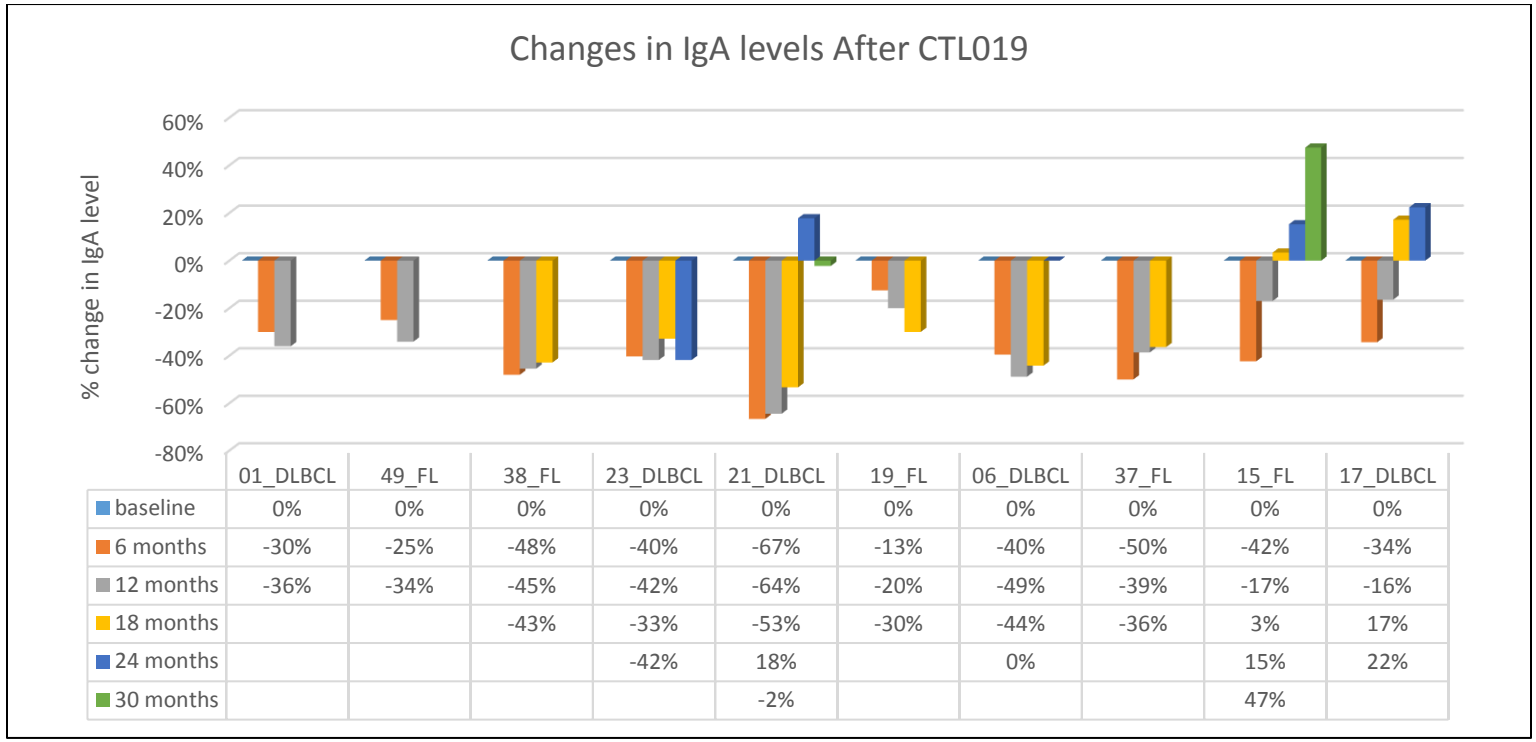
A.



	01_DLBCL	49_FL	38_FL	23_DLBCL	21_DLBCL	19_FL	06_DLBCL	37_FL	15_FL	17_DLBCL
baseline	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
month 6	-20%	-21%	-27%	-22%	-35%	10%	-23%	-5%	7%	9%
month 12	-32%	-25%	-37%	-27%	-40%	4%	-4%	-5%	17%	6%
month 18			-27%	-31%	-37%	0%	-1%	45%	8%	38%
month 24				-14%	-18%		1%		3%	75%
month 30					-16%				21%	

Figure S4: Changes in immunoglobulin levels over time in 10 patients in complete remission who were not receiving intravenous immunoglobulin.

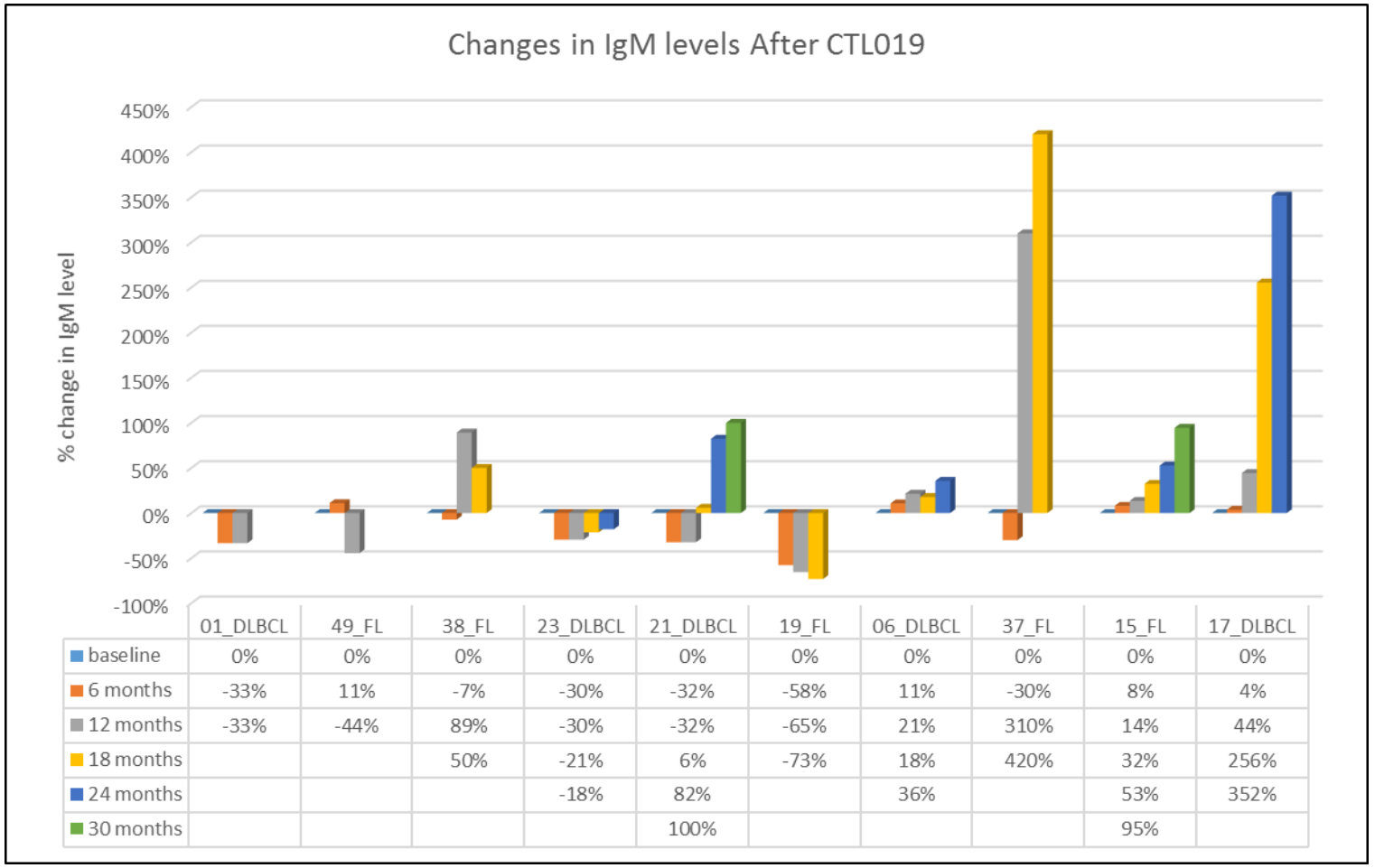
B.



	01_DLBCL	49_FL	38_FL	23_DLBCL	21_DLBCL	19_FL	06_DLBCL	37_FL	15_FL	17_DLBCL
baseline	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
6 months	-30%	-25%	-48%	-40%	-67%	-13%	-40%	-50%	-42%	-34%
12 months	-36%	-34%	-45%	-42%	-64%	-20%	-49%	-39%	-17%	-16%
18 months			-43%	-33%	-53%	-30%	-44%	-36%	3%	17%
24 months				-42%	18%		0%		15%	22%
30 months					-2%				47%	

Figure S4: Changes in immunoglobulin levels over time in 10 patients in complete remission who were not receiving intravenous immunoglobulin.

C.



	01_DLBCL	49_FL	38_FL	23_DLBCL	21_DLBCL	19_FL	06_DLBCL	37_FL	15_FL	17_DLBCL
baseline	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
6 months	-33%	11%	-7%	-30%	-32%	-58%	11%	-30%	8%	4%
12 months	-33%	-44%	89%	-30%	-32%	-65%	21%	310%	14%	44%
18 months			50%	-21%	6%	-73%	18%	420%	32%	256%
24 months				-18%	82%		36%		53%	352%
30 months					100%				95%	

Figure S5: Changes in B-cell counts and immunoglobulin levels over time in 10 patients in complete remission who were not receiving intravenous immunoglobulin.

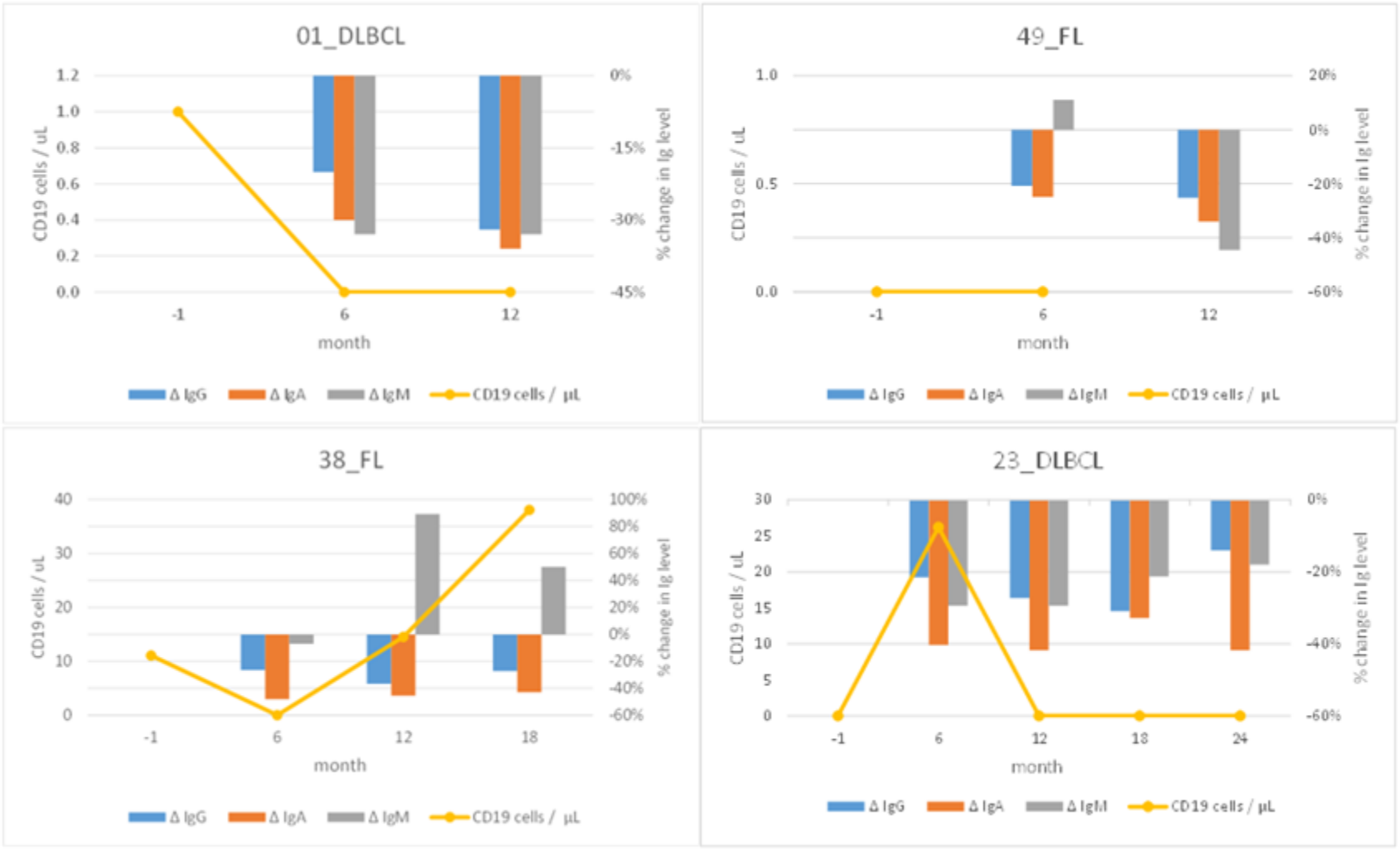


Figure S5: Changes in B-cell counts and immunoglobulin levels over time in 10 patients in complete remission who were not receiving intravenous immunoglobulin.

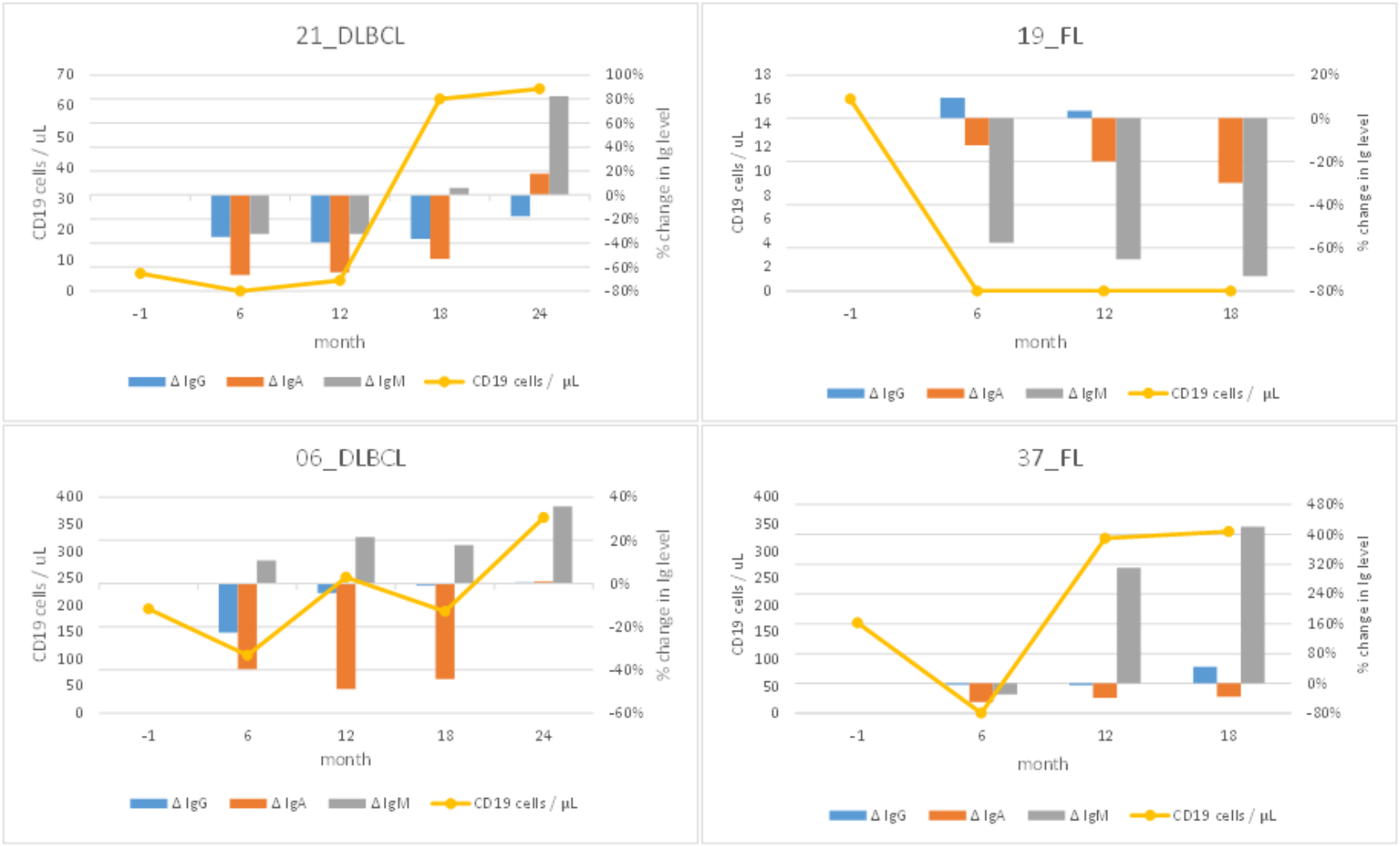


Figure S5: Changes in B-cell counts and immunoglobulin levels over time in 10 patients in complete remission who were not receiving intravenous immunoglobulin.

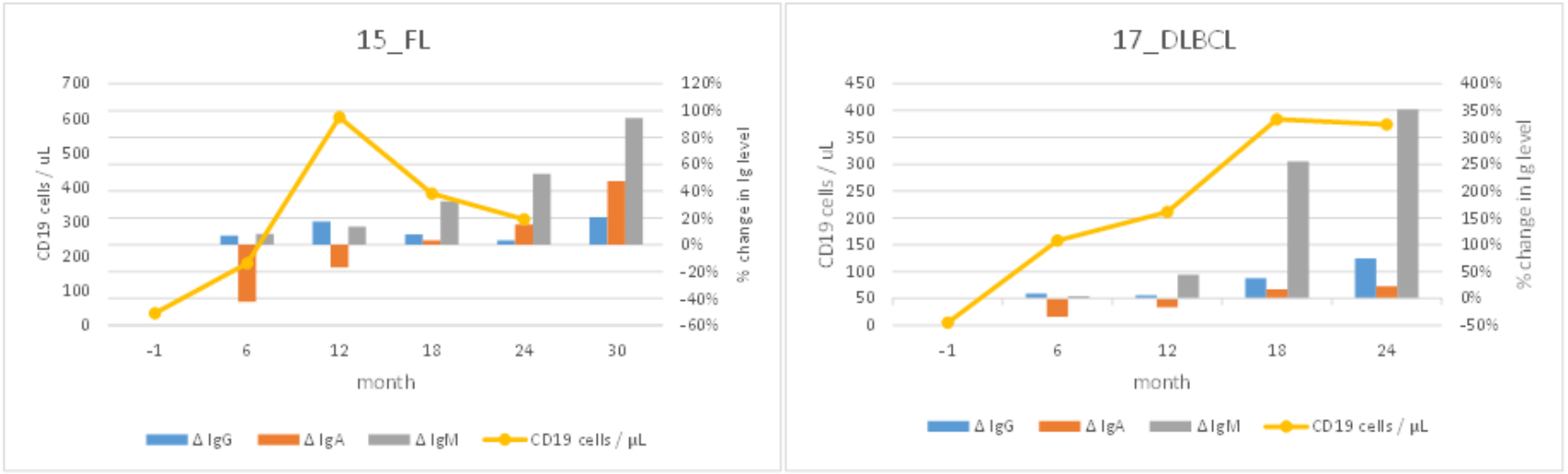
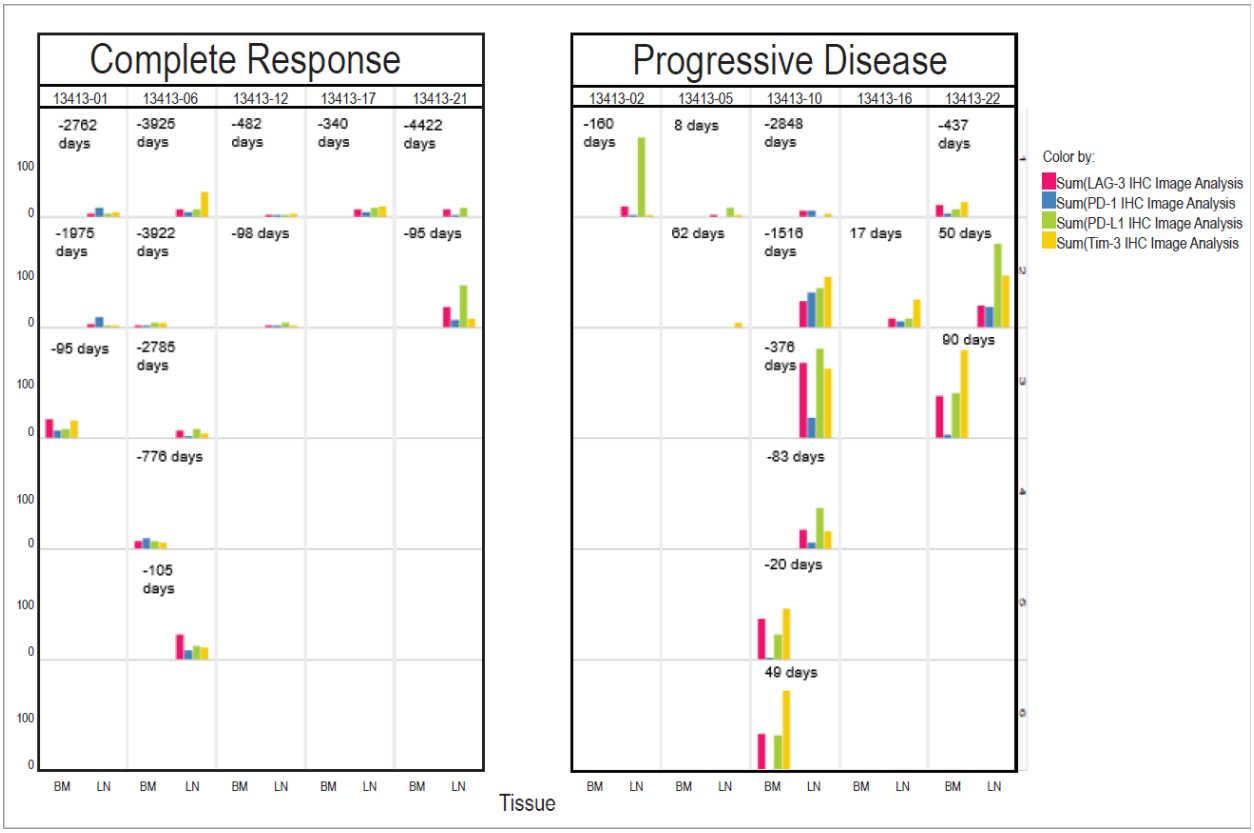


Figure S6: Immune-checkpoint analysis in lymph node and bone marrow samples from patients with diffuse large B cell lymphoma (DLBCL): PD-L1, PD1, LAG3, and TIM3 expression by patient and clinical response. Days are relative to date of CTL019 infusion.



Expression of immune-checkpoint proteins in serial lymph node and bone marrow biopsies was assessed by immunohistochemical staining and quantitative image analysis (Aperio™, Leica Biosystems Inc., Buffalo Grove, IL). Stained tissue sections were reviewed by a pathologist and digitally scanned.

Immunohistochemical Tissue Analysis: Immunohistochemical staining of formalin fixed paraffin embedded DLBCL and FL tissues were performed on a Leica Bond™ instrument using the Bond Polymer Refine Detection System according to standard methods. In brief, the slides were heat-treated for antigen retrieval in 10-mM citrate buffer and sections were incubated with the diluted primary antibodies against the checkpoint proteins listed in Figure S6. Heat-induced epitope retrieval was done for 20 minutes with ER1 solution (Leica).

Image Analysis: Immunostained slides were digitally scanned. Region(s) of interest (ROI) were defined by pathologist review. An image analysis algorithm provided quantitative expression data related to the protein of interest based on percent positive pixel counts of marker of interest (in the defined ROI) and level of biomarker expression (weak, moderate, strong). The image analysis score output is based on the biomarker expression in both non-tumor (e.g., immune cells) and tumor (lymphoma) cells in the ROI.